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(71) Applicants *(for all designated States except US)*: **ABGENIX, INC.** [US/US]; 6701 Kaiser Drive, Fremont, CA 94555 (US). **IMMUNEX CORPORATION** [US/US]; One Amgen Center Drive, Thousand Oaks, CA 91320 (US).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: **BACUS, Sarah, S.**

[US/US]; 1222 Indian Trail, Hinsdale, IL 60521 (US). **LOCKBAUM, Pamela** [US/US]; 530 Kelmore Street, Moss Beach, CA 94038 (US). **SCHWAB, Gisela** [DE/US]; 25530 Dario Terrace, Hayward, CA 94541 (US). **YANG, Xiao-dong** [CN/US]; 2833 Bryant Street, Palo Alto, CA 94306 (US). **LYNCH, David Haskett** [US/US]; 14180 N. Madison Avenue NE, Bainbridge Island, WA 98110 (US).

(74) Agent: **WILLIAMS, Andrew, W.;** McDonnell Boehnen Hulbert & Berghoff, Suite 3200, 300 South Wacker Drive, Chicago, IL 60606 (US).

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[Continued on next page]

(54) Title: METHOD FOR PREDICTING RESPONSE TO EPIDERMAL GROWTH FACTOR RECEPTOR-DIRECTED THERAPY

Example of Sample with Elevated HER1, HER3, and pERK
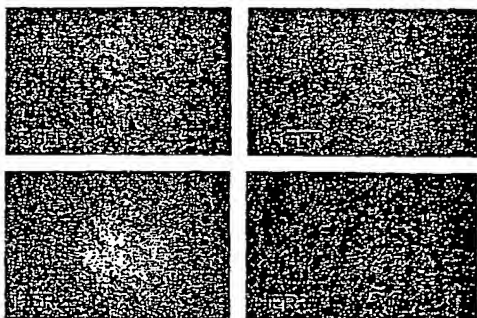
(57) Abstract: The invention provides methods for determining or predicting response to cancer therapy in an individual.

marker	level
EGFR	28
pEGFR	23
HER2	5
HER3	15
pERK	600

WO 2004/000102 A2

METHOD FOR PREDICTING RESPONSE TO EPIDERMAL GROWTH FACTOR RECEPTOR-DIRECTED THERAPY

This application claims the benefit of priority to U.S. Provisional Application Serial No. 60/389,796, filed June 19, 2002.

BACKGROUND OF THE INVENTION

1.   Field of the Invention

This invention relates to methods for predicting the response to cancer therapy in an individual.

2.   Background of the Invention

Cellular growth and differentiation processes involve growth factors that exert their actions through specific receptors expressed in the surfaces of responsive cells. Ligands binding to surface receptors, such as those that carry an intrinsic tyrosine kinase activity, trigger a cascade of events that eventually lead to cellular proliferation and differentiation (Carpenter et al., Biochem., 48: 193-216, 1979; Sachs et al., Cancer Res., 47: 1981-1986, 1987). Receptor tyrosine kinases can be classified into several groups on the basis of sequence similarity and distinct features. One of these groups includes the epidermal growth factor receptor family, which included erbB-1 (EGFR or HER-1) (Carpenter et al., Biochem., 48: 193-216, 1979); erbB-2 (HER-2/neu) (Semba et al., Proc. Natl. Acad. Sci., 82: 6497-6501, 1985; Coussens et al., Science, 230: 1130-1139, 1985, Bargmann et al., Cell, Vol. 45, 649-657, 1986); erbB-3 (HER-3) (Kraus et al., Proc. Natl. Acad. Sci., 86: 9193-9197, 1989; Carraway et al., R.A. J. Biol. Chem., 269: 14303-14306, 1994), and erbB-4 (HER-4) (Plowman et al., Nature, 366: 473-475, 1993; Tzahar et al., Biol. Chem., 269: 25226-25233, 1994).

As an example of a ligand that can bind to surface receptors, NDF (neu differentiation factor)/Heregulin is a receptor tyrosine kinase ligand that can stimulate the tyrosine phosphorylation of erbB-2 through heterodimerization with its receptors erbB-3 or erbB-4 (Peles, et al., Cell, 69:205-216, 1992, Peles, et al., EMBO J. Mar;12(3):961-71. 1993; Holmes et al, Science, 256:1205-1210, 1992. Tzahar et al., Biol. Chem., 269: 25226-25233, 1994; Plowman et al., Nature, 366: 473-475, 1993; Pinkas-Kramarski et al., Proc. Natl. Acad. Sci., 91:9387-9391, 1994; Pinkas-Kramarski et al., The Journal of Biological Chemistry, Vol. 271, No. 32: 19029-19032, 1996; Pinkas-Kramarski et al.,

Oncogene, 16, 1249-1258, 1998.). Depending on the cell line studied, NDF/Heregulin can either elicit a growth arrest and differentiation phenotype, resulting in morphological changes, induction of lipids, and expression of intracellular adhesion molecule-1, or induce a mitogenic response (Holmes *et al.*, Science, 256:1205-1210, 1992; Peles *et al.*,

5   Cell, 69:205-216, 1992; Bacus *et al.*, Cancer Res. 53:5251-5261, 1993).

Activation of erbB receptor heterodimers is coupled to and stimulates downstream MAPK-Erk1/2 and PI3K-AKT growth and survival pathways whose deregulation in cancer has been linked to disease progression and refractoriness to therapy (Olayioye, M.A., *et al.*, *Mol. Cell. Biol.* **18**, 5042-5051 (1998), Fukazawa, T., *et al.*, *J. Biol. Chem.*

10 **271**, 14554-14559 (1996), Hackel, P.O., *et al.*, *Curr. Opin. Cell Biol.* **11**, 184-189 (1999); Tzahar, E., *et al.*, *Mol. Cell. Biol.* **16**, 5276-5287 (1996); Lange, C.A., *et al.*, *J. Biol. Chem.* **273**, 31308-31316 (1998). For example, HER-3 is a major docking site for phoshoinositide-3-kinase (PI3K). In addition, NDF/Heregulin stimulation causes activation of the PI3K pathway and phosphorylation of AKT (Altiok *et al.*, J. Biol.

15 Chem., 274, 32274-32278, 1999; Liu *et al.*, Res. Comm., 261, 897-903, 1999; Xing *et al.*, Nature Med., 6, 189-195, 2000). These observations implicate PI3K/AKT in the signaling cascade that results from HER-3 heterodimerization with overexpressed HER-2/neu receptors in breast cancer cells; activation of PI3K/AKT promote cell survival and enhanced tumor aggressiveness (Shak, Semin. Oncol., Suppl 12:71-77, 1999; Huang *et*

20 *al.*, Clinical Cancer Res., Vol. 7: 2166-2174, 2000). In addition, AKT2 was reported to be activated and overexpressed in HER-2/neu-overexpressing breast cancers (Bacus *et al.*, Oncogene, 21: 3532-3540, 2002).

Most tumors of epithelial origin express multiple erbB (HER) receptors and co-express one or more EGF-related ligands suggesting that autocrine receptor activation

25 plays a role in tumor cell proliferation. Because these ligands activate different erbB/HER receptors, it is possible that multiple erbB receptor combinations might be active in a tumor, a characteristic that could influence its response to an erbB-targeted therapeutic. For example, erbB-2/HER-2 is overexpressed in 20 to 30% of all breast cancers, and its overexpression is associated with poor prognosis, suggesting that it could

30 be used as a target for anti-tumor agents (Slamon et al., Science, 235: 177-182, 1987; Tagliabue et al., Int. J. Cancer, 47: 933-937, 1991; Hudziak et al., Mol. Cell. Biol., 9: 1165-1172, 1989). Studies have shown that in erbB-2 overexpressing breast cancer cells, treatment with antibodies specific to HER-2/erbB-2 in combination with chemotherapeutic agents (e.g., cisplatin, doxoubicin, taxol) elicits a higher cytotoxic

response than treatment with chemotherapy alone (Hancock et al., Cancer Res., 51: 4575-4580, 1991; Arteaga et al., Cancer, 54:3758-3765, 1994; Pietras et al., Oncogene, 9: 1829-1838, 1994). One possible mechanism by which HER-2/erbB-2 antibodies might enhance cytotoxicity to chemotherapeutic agents is through the modulation of the HER-

5   2/erbB-2 protein expression, (Bacus et al., Cell Growth & Diff., 3: 401-411, 1992, Bacus et al., Cancer Res. 53:5251-5261, 1993; Stancovski et al., Proc Natl Acad Sci USA 88: 8691-8695, 1991; Klapper et al., Oncogene 14, 2099-2109, 1997, and Klapper et al., Cancer Res., 60: 3384-3388, 2000), or by interfering with DNA repair (Arteaga et al., Cancer, 54:3758-3765, 1994, and Arteaga et al., J Clinical Oncology, Vol. 19, No 18s ,

10   32s-40s, 2001; Pietras et al., Oncogene, 9: 1829-1838, 1994).

Because of the effect of anti-HER-2/erbB-2 antibodies on cellular growth, a number of approaches have been used to therapeutically target HER-2/erbB-2 or EGFR overexpressing cancers. For clinical use, one approach is to interfere with the kinase activity of the receptor by using inhibitors that block the nucleotide binding site of HER-

15   2/neu or EGFR (Bruns, et al., Cancer Research, 60,2926-2935, (2000); Christensen, et al, Clinical Cancer Research, Vol. 7, 4230-4238, 2001, Erlichman, et al., Cancer Research 61, 739-748, 2001, Fujimura, et al., Clinical Cancer Research, Vol. 8, 2448-2454, 2002; Herbst, et al., Journal of Clincal Oncology, Vol. 20, No. 18, 3815-3825, 2002; Hidalgo, et al, J. Clinical Oncology, Vol 19, No 13: pp 3267-3279, 2001; Moasser, et al, Cancer Res.,

20   61: 7184-7188, 2001; Normanno, et al, Ann. of Oncol., 13: 65-72, 2002). A second approach is using ansamycins to influence the stability of HER2/neu receptors (Munster, et al., Cancer Research 62, 3132-3137, 2002; Basso et al, Oncogene, 21: 1159-1166, 2002). Another approach is the use of antibodies directed to various erbB receptors specifically EGFR or HER-2/neu (Alaoui-Jamali, et al Biochem. Cell. Biol., 75:315-325,

25   1997; Albanell, et al., J. National Cancer Institute, Vol 93, No. 24, 1830-31, 2001; Baselga, et al., Pharmacol Ther 64: 127-154, 1994 and Baselga, et al., Annuals of Oncology 13: 8-9, 2002; Mendelsohn, Seminars in Cancer Biology, Vol. 1, pp. 339-344, 1990). A number of monoclonal antibodies and small molecule, tyrosine kinase inhibitors targeting EGFR or erbB-2 have been developed. For example, HERCEPTIN®

30   is approved for treating the 25% of women whose breast cancers overexpress erbB-2 protein or demonstrate erbB-2 gene amplification (Cobleigh, M.A., et al., J. Clin. Oncol. 17, 2639-2648 (1999)). Analysis of various antibodies to HER-2/neu has led to the identification of the murine monoclonal, 4D5. This antibody recognizes an extracellular epitope (amino acids 529 to 627) in the cysteine-rich II domain that resides very close to

the transmembrane region. Treatment of breast cancer cells with 4D5 partially blocks NDF/heregulin activation of HER-2-HER-3 complexes, as measured by receptor phosphorylation assays. To allow for chronic human administration, murine 4D5 was humanized to generate HERCEPTIN® (trastuzumab) (Sliwkowski et al, Sem. in Oncol.,

5   26:60-70, 1999; Ye et al., Oncogene, 18: 731-738, 1999; Carter et al, Proc. Natl Acad Sci USA 89:4285-4289, 1992; Fujimoto-Ouchi et al, Cancer Chemother Pharmacol, 49: 211-216, 2002; Vogel, et al., Oncology, 61(suppl 2):37-42, 2001; Vogel, et al., Journal of Clinical Oncology, Vol 20, No. 3:719-726, 2002). In addition, several EGFR-targeted therapies are currently under clinical investigation (Mendelsohn, J., & Baselga, J.,

10   *Oncogene* 19, 6550-6565 (2000); Xia, W., *et al. Oncogene* 21, 6255-6263 (2002)). In particular, a human anti-EGFr monoclonal antibody, designated ABX-EGF (and also referred to herein as ABX-0303, as described in detail in U.S. Patent No. 6,235,883; the disclosure of which is hereby incorporated by reference), is being developed by Abgenix, Inc. and Immunex Corporation (Yang X et al. Development of ABX-EGF, a fully human

15   anti-EGF receptor monoclonal antibody, for cancer therapy. Crit Rev Oncol Hemato 38(1):17-23 (2001); Yang X-D et al. Eradication of Established Tumors by a Fully Human Monoclonal Antibody to the Epidermal Growth Factor Receptor without Concomitant Chemotherapy. Cancer Research 59(6):1236-1243 (1999)).

     Historically, cytotoxic cancer therapies have been developed based on maximum

20   tolerated doses (MTD), treating patients without understanding the tumor profile for likely responders. Hence, patients were often subjected to toxic therapies with limited therapeutic benefit. Recently, elucidating tumor growth and survival pathways has led to the development of tumor-targeted therapies. An example of this approach is Gleevec™, an inhibitor of the c-abl family of tyrosine kinases approved for treating chronic myeloid

25   leukemia and gastrointestinal stromal tumors (Druker, B.J. *et al., N. Engl. J. Med.* 344, 1031-1037 (2001); Demitri, G.D., *et al.; N. Engl. J. Med.* 347, 472-480 (2002)).

     In contrast, most erbB-receptor targeted therapies primarily exert cytostatic anti-tumor effects, necessitating their chronic administration. Identification of biologically effective doses (BED), the dose or dose range that maximally inhibits the intended target,

30   beyond which dose escalation is likely to add toxicity without benefit, is therefore essential. Moreover, many of these agents will be used in combination with cytotoxic therapies, where added toxicity may not be tolerable, further supporting BED-based dosing. Targeted-therapy implies that populations of likely responders exists, and can be identified.

In view of the severe and deleterious consequences of administering an inappropriate or ineffective therapy to a human cancer patient, there exists a need in the art for predicting the response to cancer therapy in an individual.

5   **SUMMARY OF THE INVENTION**

This invention provides methods for predicting a response of an individual to a particular cancer treatment regimen.

In a first aspect, the invention provides methods for predicting a response to an epidermal growth factor receptor-directed therapy in a human subject, the method

10   comprising the step of assaying a tumor sample from the human subject before therapy with one or a plurality of reagents that detect expression and/or activation of predictive biomarkers for cancer; and determining a pattern of expression and/or activation of at least two of said predictive biomarkers, wherein the pattern predicts the human subject's response to the epidermal growth factor receptor-directed therapy.   In certain

15   embodiments, the predictive biomarker is a growth factor receptor, or a growth factor receptor-related downstream signaling molecule. The growth factor receptors can be HER1 (EGFR), pHER1, HER2/neu, HER3, or any combination thereof. The growth factor receptor-related downstream signaling molecules can be pERK.   In further embodiments, the predictive biomarkers are HER1 (EGFR), pHER1, HER2/neu, HER3,

20   or pERK, or any combination thereof.

In further embodiements, the predictive biomarkers are HER1 (EGFR) and HER3. In other embodiments, when HER1 (EGFR) is undetectable is predictive of the human subject not responding to the epidermal growth factor receptor-directed therapy. In still other embodiments, wherein when HER3 is undetectable is predictive of the human

25   subject responding to the epidermal growth factor receptor-directed therapy. In further embodiments, the predictive biomarkers are HER1 (EGFR) and pERK; or the predictive biomarkers are pERK and HER3, or the predictive biomarkers are HER1 (EGFR), HER3, and pERK.

In a second aspect, the invention provides a kit for the determining a response to

30   an epidermal growth factor receptor-directed therapy in a subject, wherein the kit comprises at least two reagents that detect expression and/or activation of predictive biomarkers for cancer. In certain embodiments, the kit comprises three reagents. In other embodiments, the predictive biomarkers are HER1, HER3, or pERK, or any combination thereof.

In a third aspect, the invention provides methods for predicting a response to a cancer therapy in a human subject, the method comprising the step of assaying a cell or tissue sample from the human subject before therapy with one or a plurality of reagents that detect expression and/or activation of predictive biomarkers for cancer, wherein said predicative biomarkers consist of growth factor receptor ligands; and determining a pattern of expression and/or activation of at least two of said predictive biomarkers, wherein the pattern predicts the human subject's response to the cancer therapy. In other embodiments, the growth factor receptors are HER1 (EGFR), pHER1, HER2/neu, HER3 or any combination thereof. In still other embodiments, the cancer therapy is an epidermal growth factor receptor-directed therapy. In further embodiments, the cancer therapy is an anti-EGFR antibody. Further, the antibody is ABX-0303.

In a fourth aspect, the invention provides methods of selecting a subject with cancer for treatment with a molecule targeting epidermal growth factor receptor (EGFR), comprising determining the level of expression of HER3 in a cell or tissue sample from the subject, wherein if the level of HER3 expression is low in the cells, the subject is selected. In other embodiments, the molecule is an anti-EGFR antibody. Further, the antibody is ABX-0303. In still other embodiments, the determining step further comprises determining expression of one or more of HER1 (EGFR), pHER1, HER2/neu, and pERK.

In a fifth aspect, the invention provides method of predicting the likely response rate to a molecule targeting epidermal growth factor receptor (EGFR) of a subject having a cancer that overexpresses EGFR, comprising the step of determining the level of expression of HER3 in a cell or tissue sample from the subject, wherein if the level of HER3 expression is low in the cells, the subject is likely to respond to the molecule targeting EGFR. In other embodiments, the molecule is an anti-EGFR antibody. Further, the antibody is ABX-0303. In still other embodiments, the determining step further comprises determining expression of one or more of HER1 (EGFR), pHER1, HER2/neu, and pERK.

In a sixth aspect, the invention provides methods of treating a subject with cancer, comprising determining the level of expression of HER3 in the cells from the subject, and treating the subject with an anti-EGFR antibody when HER3 expression levels in the cell are low. In further embodiments, the antibody is ABX-0303. In other embodiments, the determining step further comprises determining expression of one or more of HER1 (EGFR), pHER1, HER2/neu, and pERK. Further, the antibody is ABX-0303. In still

other embodiments, the level of expression of HER3 is undetectable. Further, the antibody is ABX-0303.

In a seventh aspect, the invention provides methods of selecting a subject with cancer for treatment with a molecule targeting epidermal growth factor receptor (EGFR), the method comprising:

a)   determining an expression and/or activation profile of two or more growth factor receptors in cells and/or tissues of the subject; and

b)   selecting the subject based on the expression and/or activation profile, wherein the subject is selected when the level of expression of HER3 is low, the level of expression of the HER1 is high, and/or the level of the pERK index is high. In other embodiments, the molecule is an anti-EGFR antibody. Further, the antibody is ABX-0303. In another aspect, the growth factor receptors comprise one or more of HER1 (EGFR), pHER1, HER2/neu, and HER3.

Specific embodiments of the present invention will become evident from the following more detailed description of certain preferred embodiments and the claims.

DESCRIPTION OF THE DRAWINGS

Figures 1 illustrates the response to ABX-0303 by a patient with elevated HER1 and pERK, and decreased levels of HER3. The figure represents quantitative immunohistochemical analysis of EGFR, pEGFR, HER2, HER3, and pERK.

Figure 2 illustrates the response to ABX-0303 by a patient with elevated HER1, HER3, and pERK. The figure represents quantitative immunohistochemical analysis of EGFR, pEGFR, HER2, HER3, and pERK.

DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

This invention provides methods for predicting response in cancer subjects to cancer therapy, including human cancer patients.

In contrast to traditional anticancer methods, where chemotherapeutic drug treatment is undertaken as an adjunct to and after surgical intervention, neoadjuvant (or primary) chemotherapy consists of administering drugs as an initial treatment in cancer patients. One advantage of such an approach is that, for primary tumors of more than 3 cm, it permits the use of conservative surgical procedures (as opposed to, *e.g.*, radical mastectomy in breast cancer patients) for the majority of patients, due to the tumor-

shrinking effect of the chemotherapy. Another advantage is that for many cancers, a partial and/or complete response is achieved in about two-thirds of all cases. Finally, because the majority of patients are responsive after two to three cycles of chemotherapeutic treatment, it is possible to monitor the *in vivo* efficacy of the
5   chemotherapeutic regimen employed, which is important for a timely identification of those cancers which are non-responsive to chemotherapeutic treatment. Timely identification of non-responsive tumors, in turn, allows the clinician to limit the cancer patient's exposure to unnecessary side-effects of treatment and to institute alternative treatments. However, the methods present in the art, including histological examination,
10 are insufficient for such a timely and accurate identification. The present invention provides methods by which a more informed and effective regime of therapy can be administered.

A cancer diagnosis, both an initial diagnosis of disease and subsequent monitoring of the disease course (before, during, or after treatment) is conventionally confirmed
15 through histological examination of cell or tissue samples removed from a patient. Clinical pathologists need to be able to accurately determine whether such samples are benign or malignant and to classify the aggressiveness of tumor samples deemed to be malignant, because these determinations often form the basis for selecting a suitable course of patient treatment. Similarly, the pathologist needs to be able to detect the extent
20 to which a cancer has grown or gone into remission, particularly as a result of or consequent to treatment, most particularly treatment with chemotherapeutic or biological agents.

Histological examination traditionally entails tissue-staining procedures that permit morphological features of a sample to be readily observed under a light
25 microscope. A pathologist, after examining the stained sample, typically makes a qualitative determination of whether the tumor sample is malignant. It is difficult, however, to ascertain a tumor's aggressiveness merely through histological examination of the sample, because a tumor's aggressiveness is often a result of the biochemistry of the cells within the tumor, such as protein expression or suppression and protein
30 activation, which may or may not be reflected by the morphology of the sample. Therefore, it is important to be able to assess the biochemistry of the cells within a tumor sample. Further, it is desirable to observe and quantitate both gene expression and protein activation of tumor related genes or proteins, or more specifically cellular components of a tumor-related signally pathway.

Cancer therapy can be based on molecular profiling of tumors rather than histology or site of disease. Elucidating the biological effects of targeted-therapies in tumor tissue and correlating these effects with clinical response helps identify the predominant growth and survival pathways operative in tumors, thereby establishing a profile of likely responders and conversely providing a rational for designing strategies to overcoming resistance.

It is necessary to consider additional biomarkers beyond the presence of the target, such as EGFR, for subjects who are considered for treatment with, for example, biomolecules that modulate EGFR. Not all tumor cells respond to inhibition of ErbB receptors, despite exhibiting aberrant ErbB-1 and/or ErbB-2 expression. Examples include MKN7 and BT474 ErbB receptor-overexpressing carcinoma cell lines, wherein BT474 cells respond to HERCEPTIN® but MKN7 cells do not. These observations have clear implications for erbB-directed therapeutics and the consideration of the expression of multiple erbB receptors and in tumors.

For example, ABX-0303 (as referred to herein as ABX-EGF), an epidermal growth factor receptor-directed therapy sponsored by Abgenix and Immunex Corporation, effectively targets HER1 to prevent the growth of renal cell cancers. Based on the positive correlation between pERK expression and response to ABX-0303, it is likely that HER1 is acting through the MAPK pathway. In addition, HER3 was found to be elevated in a large percentage of renal biopsies analyzed from non-responders. One possibility is that HER3 is interacting with HER2 to confound the action of the drug.

Automated (computer-aided) image analysis systems known in the art can augment visual examination of samples. In a representative system, the cell or tissue sample is exposed to detectably labeled reagents specific for a particular biological marker, and the magnified image of the cell is then processed by a computer that receives the image from a charge-coupled device (CCD) or camera such as a television camera. Such a system can be used, for example, to detect and measure expression and activation levels of Her1, pHER1 HER2, HER3, and pERK in a sample. Additional biomarkers are also contemplated by this invention. This methodology provides more accurate diagnosis of cancer and a better characterization of gene expression in histologically identified cancer cells, most particularly with regard to expression of tumor marker genes or genes known to be expressed in particular cancer types and subtypes (i.e., different degrees of malignancy). This information permits a more informed and effective regimen of therapy

to be administered, because drugs with clinical efficacy for certain tumor types or subtypes can be administered to patients whose cells are so identified.

For example, expression and activation of proteins expressed from tumor-related genes can be detected and quantitated using methods of the present invention. Further,
5   expression and activation of proteins that are cellular components of a tumor-related signaling pathway can be detected and quantitated using methods of the present invention. Further, proteins associated with cancer can be quantified by image analysis using a suitable primary antibody against biomarkers, such as, but not limited to, Her-1, Her-2, p-Her-1, Her-3, or p-ERK, and a secondary antibody (such as rabbit anti-mouse
10 IgG when using mouse primary antibodies) and/or a tertiary avidin (or Strepavidin) biotin complex ("ABC").

In practicing the method of the present invention, staining procedures can be carried out by a technician in the laboratory. Alternatively, the staining procedures can be carried out using automated systems. In either case, staining procedures for use
15 according to the methods of this invention are performed according to standard techniques and protocols well-established in the art.

By "cell or tissue sample" is meant biological samples comprising cells, most preferably tumor cells, that are isolated from body samples, such as, but not limited to, smears, sputum, biopsies, secretions, cerebrospinal fluid, bile, blood, lymph fluid, urine
20 and faeces, or tissue which has been removed from organs, such as breast, lung, intestine, skin, cervix, prostate, and stomach. For example, a tissue sample can comprise a region of functionally related cells or adjacent cells.

The amount of target protein can then be quantitated by the average optical density of the stained antigens. Also, the proportion or percentage of total tissue area
25 stained may be readily calculated, as the area stained above an antibody threshold level in the second image. Following visualization of nuclei containing biomarkers, the percentage or amount of such cells in tissue derived from patients after treatment may be compared to the percentage or amount of such cells in untreated tissue or said tissue prior to treatment. For purposes of the invention herein, "determining" a pattern of expression
30 and/or activation of a biomarker is understood broadly to mean merely obtaining the information on such biomarker(s), either through direct examination or indirectly from, for example, a contract diagnostic service.

Thus, the level of expression and/or activation in a cell can be determined by, for example, quantitative immunohistochemistry. In this case, the level of expression of

HER1, HER2, and/or HER3 is considered to be low if the OD is less than 9. Further, the level of expression is also considered to be low if the OD is less than 5, or less than 3, or if the OD is 0 (undetectable). In addition, the level of expression of HER1, HER2, and/or HER3 is considered to be high is the OD is greater than 9. Further, the level of expression can be considered high for pERK when the pERK index is greater than 99.

Particularly useful embodiments of the present invention and the advantages thereof can be understood by referring to Examples 1-7. These Examples are illustrative of specific embodiments of the invention, and various uses thereof. They are set forth for explanatory purposes only, and are not to be taken as limiting the invention.

Example 1

Staining procedure for biomarkers

Human tumor tissue samples were stained as follows. Tumor tissue in 10% Neutral Buffered Formalin Paraffin blocks are sectioned at 4 microns and the sections placed onto coated slides. EGFR immunostaining is preformed by using Ventana Medical Instruments, Inc. monoclonal 111.6; HER-2 immunostaining is performed by using Ventana Medical Instruments, Inc. monoclonal CB11, and HER-3 immunostaining is performed by using Ventana Medical Instruments, Inc. monoclonal SGP1. Her-1, Her-2, and Her-3 are immunostained using, for example, the "BenchMark" (VMSI) with I-VIEW (VMSI) detection chemistry. pEGFR immunostaining is performed by using Chemicon monoclonal MB3052. p-ERK (1:100) is obtained from Cell Signaling Technology (Beverly, MA) and immunostained using a labeled streptavidin peroxidase technique.

For example, slides for p-ERK (1:100) are antigen retrieved using 0.1 M citrate buffer, pH 6.0 in the "decloaker" (Biocare Corp.) and the sections incubated overnight with the primaries at 4 °C. The next day, the slides for pERK and pAKT are placed onto the Autostainer (Dako Corp.) and the "LSAB2" kit (Dako) is employed as the detection chemistry. DAB (Dako) is used as the chromogen. After immunostaining, all immunomarkers, are counterstained manually with 4% ethyl green (Sigma).

Example 2

Procedure for Immunohistochemistry

Quantitative immunohistochemistry (IHC) is performed as previously described (Bacus, S., *et al.*, *Analyt. Quant. Cytol. Histol.* **19**, 316-328 (1997)). EGFR, and erbB-2 immunostaining is performed using the pre-diluted EGFR (Ventana monoclonal 111.6)

5 and erbB-2 (Ventana monoclonal CB11) antibodies from Ventana on the VMSI automated "BenchMark" staining module as described. The VMSI "I-View" detection kit is used for all of the VMSI pre-diluted primary antibodies. HER-3 is also immunostained using the "BenchMark" with I-VIEW detection chemistry. pErk is immunostained using a labeled streptavidin peroxidase technique. Phospho-Erk1/2 slides are antigen retrieved

10 as described (Bacus, S., *et al.*, *Analyt. Quant. Cytol. Histol.* **19**, 316-328 (1997)). Slides are placed onto the Autostainer (Dako Corp.) and the "LSAB2" kit (Dako) employed as the detection chemistry. pEGFR is immunostained in a similar labeled streptavidin peroxidase technique. pEGFR slides are antigen retrieved with 1 mM EDTA and slides for p-erbB-2 with 0.1M citrate buffer, pH 6.0, in the "decloaker". After staining, EGFR,

15 HER2, HER3, pErk, and pEGFR, are counterstained manually with 4% ethyl green (Sigma). TUNEL assay (Roche Diagnostics, Indianapolis) is performed according to the manufacturer's instructions. Investigators preparing and analyzing tissue sections are blinded to both patient tumor type and response to therapy.

For IHC, antibodies to EGFR, HER2 and HER3 were from Ventana Medical

20 Scientific Instruments (VMSI) (Tucson, AZ); pERK was from Cell Signaling Technology Inc. (Beverly, MA); anti pEGFR and from Chemicon (Temecula, CA).

Example 3

25 ## Analysis of treatment with an Epidermal Growth Factor-Directed Therapy

53 samples from renal cancer patients enrolled in a clinical trial sponsored by Abgenix and Immunex Corporation for an investigational drug directed to EGFR were analyzed for expression of various biomarkers. The sample slides were obtained from

30 Impath Laboratories, Inc.

Immunohistochemical (IHC) analyses were carried out using the automated staining devices as described above. The antibodies used for the specific biomarkers included: Ventana monoclonal 111.6 for EGFR, Chemicon monoclonal MB3052 for pEGFR, polyclonal pERK from Cell Signaling Technology for pERK, Ventana

monoclonal SGP1 for HER3, and Ventana monoclonal CB11 for HER2. For each specimen, a slide was stained with control mouse immunoglobulins to establish the existence and localization of background staining. In addition, appropriate positive controls were run for each IHC stain. Following counterstaining with ethyl green, the
5   slides were permanently mounted and analyzed using interactive image analysis to establish the optical density of peroxidase stained cytoplasmic and membrane staining. In the case of pERK, the fraction of cells expressing nuclear pERK, and the intensity of the stain were measured using a CAS system, and the results were expressed as the pERK index (product of OD x percent positive nuclear area). The technician quantifying the
10   results observed areas of tumor that were not adjacent to normal renal tubules to avoid confounding the quantification. In all cases, the stained slides were viewed by at least two people, including a pathologist and a senior scientist, to establish that the quantification results were representative of the stained sections.

Immunohistochemical analyses, quantification, and correlation with response data
15   were completed for twenty-nine (29) of the specimens. Partial data, representing analysis of only a subset of the selected biomarkers, was available for an additional twelve (12) samples. No data was obtained on the remaining specimens because of questions as to the identity of the slides, or the absence of information concerning the patient's response to ABX-0303. The conclusions that can be drawn from the analysis include, but are not
20   limited to, that response to ABX-0303 is related to the expression of HER1, and that elevated expression of HER3 compromises the action of the drug.

Results of the IHC analysis of the renal cancer biopsies, for which at least HER1 IHC results and clinical response information was available, is presented in Table 1.

TABLE I

IHC ANALYSIS

Pt #	HER1	HER1 "score"	pHER1	pERK Index	HER3-st	HER2-st	HER2 cocktail	Treatment Group	Histologic Type	Response
3001	8	0		36	17	3		1.0 mg/kg	Clear Cell Carcinoma	PD
3002	19	+2	20	7	27	5	+2	1.0 mg/kg	Clear Cell Carcinoma	S
3003	8		2					1.0 mg/kg	Other	MR
3006	6	+1	0	896	32	0	+1 (focal)		Clear Cell Carcinoma	PD
3007	18	+2	6	1127	27	0	0	1.0 mg/kg	Clear Cell Carcinoma	S
3008	19	+2	0	12	15	3		1.0 mg/kg	Clear Cell Carcinoma	PD
3009	0	+2 (focal)	0	96	15	0	0	1.0 mg/kg	Clear Cell Carcinoma	PD
3010	21	+3	0	1100	33	10				PD
3011	5		0					1.0 mg/kg	Clear Cell Carcinoma	S
3012	8	+1	3	40	15	1	0	1.0 mg/kg	Clear Cell Carcinoma	S
3014	17	+2 to +3		132	0			1.0 mg/kg	Other	S
3018	20	+3	9	90	16	0	0	1.0 mg/kg	Papillary Carcinoma	PD
3019	18	+2	14	924	2	0		1.0 mg/kg	Other	PR

3020	10	+3	3	1176	14	13		1.0 mg/kg	Clear Cell Carcinoma	S
3031	0	0	5	0	0	0	+1	1.5 mg/kg	Clear Cell Carcinoma	PD
3032	0	+1 (very weak)	1	99	2	0	+1	1.5 mg/kg	Clear Cell Carcinoma	S
3033	18	+2 to +3	14	390	50	2	+2			PD
3036	4	+1	0	540	0	0	+1			S
3037	8							1.5 mg/kg	Clear Cell Carcinoma	S
3039	16	+2	0	208	21			1.5 mg/kg	Clear Cell Carcinoma	S
3043	15	+2	0	143	40	0	+2	1.5 mg/kg	Papillary Carcinoma	PD
3047	11	+1 to +2 (focal)	0	64	37	0	+1	1.5 mg/kg	Clear Cell Carcinoma	S
3051	18	+2	5	247				1.5 mg/kg	Clear Cell Carcinoma	S
3053	7	+1	26	0	45	2		1.5 mg/kg	Other	PD
3065	23	+3	15	108	22			2.0 mg/kg	Clear Cell Carcinoma	S
3068	16	+2	10	221	4	9	+1	2.0 mg/kg	Clear Cell Carcinoma	S
3070	33	+3	0	465	38	15		2.0 mg/kg	Clear Cell Carcinoma	PD
3073	2	0	0	126	23	0		2.0 mg/kg	Papillary Carcinoma	PD
3075	22	+3	4	77	28	13		2.0 mg/kg	Clear Cell Carcinoma	PD
3077	25		0					2.0 mg/kg	Clear Cell Carcinoma	S
3078	6	0	1	90	11			2.0 mg/kg	Clear Cell Carcinoma	PD
3080	8	+1 (weak)	21	8	44	11		2.0 mg/kg	Clear Cell Carcinoma	PD
3084	20	+3	0	8	34	5		2.0 mg/kg	Clear Cell Carcinoma	S
3092	6	+1	5	576				2.5 mg/kg	Clear Cell Carcinoma	S
3095	10	+1	1	0	9	0	+2			MR
3099	13	+2	34	90	13	6		2.5 mg/kg	Clear Cell Carcinoma	S
3101	11	+2 to +3	0	6	25	0	0	2.5 mg/kg	Other	PD
3103	20	+3	0	1134	30	5	+2	2.5 mg/kg	Clear Cell Carcinoma	PD
3105	15							2.5 mg/kg	Papillary Carcinoma	PD
3108	11							2.5 mg/kg	Clear Cell Carcinoma	S

Results are presented as OD unless otherwise indicated.

Based on this analysis, in which the positive and negative predictive values were calculated as a function of the optical density, or fraction positivity, values were determined to stratify samples based upon expression of the biomarkers analyzed. The results of the analysis of using these stratification criteria is presented in Table II.

TABLE II

DATA ANALYSIS

group of samples (n)	RESPONDERS	NONRESPONDERS
all reported in study (41)	56%	44%
HER1 OD>9 (25)	60%	40%
HER1 OD<10 (16)	44%	56%
HER1 visual score of +1 or greater (30)	60%	40%
pERK index>99 (19)	63%	37%
pERK index<100 (16)	38%	62%
HER1+/perk- (8)*	50%	50%
HER1+/perk+ (12)	64%	36%

HER3 OD>9 (26)	38%	62%
HER3 OD<10 (7)	86%	14%
HER3+/HER1+ (17)	47%	53%
HER3+HER1- (9)	22%	78%
HER3+/perk+ (13)	46%	54%
HER3+/perk- (13)	31%	69%
HER2 OD>9 (6)	33%	67%
HER2 OD<10 (23)	48%	52%

* for purposes of this analysis "+" refers to OD greater than 9 upon quantification of HER1, HER2, or HER3; or pERK index of greater than 99.

Overall, there was no single marker that, when quantified, absolutely correlated with response to ABX-0303. This data indicates, however, that expression of HER1 and pERK predict response to the drug, while samples expressing HER3 are less likely to respond well. The quantitative analysis presented assumes that any expression of these markers that gives an optical density reading of 10 or greater was significant. It is interesting to note that visual assessment of HER1 staining, where any intensity of 1+ or greater is considered positive, agrees with the quantification of this marker. Also of interest, only three of the thirty-three samples examined by a pathologist were scored as "0" for HER1 staining intensity, and all three samples were from patients who failed to respond to ABX-0303. Thus, the absence of detectable HER1 (staining intensity "0"), can also be a predictor of response to ABX-0303.

The presence of HER3 seems to be a negative predictor of response. Patients whose specimens lacked HER3 by the criterion used here were more likely to respond than those that had HER3 (86% vs. 38%). There was no significant correlation between the presence of the phosphorylated form of HER1 and response to ABX-0303. The lack of pHER1 expression, however, even in samples with significantly elevated levels of HER1, may have been a result of a failure to preserve the phosphorylated form of this protein during the collection and processing of biopsies. Only 6 of the samples analyzed by quantitative IHC were HER2 "positive" by the criterion of having on OD of 10 or greater. As shown in Table II, these were predominantly poor responders to the drug. Interestingly, all six of these samples had elevated levels of HER3. HER2 expression, quantified using a monoclonal antibody directed against the external domain of HER2, was further determined using a cocktail of antibodies that recognize both the internal and

external domains of the protein. While some additional samples appeared to be positive using this alternate approach, these observations were not sufficient to confirm the correlation between HER2 and HER3 expression with lack of response to ABX-0303.

5   The expression and co-expression of HER1, HER3, and pERK indicates that HER1, acting through pERK, was critical to ABX-0303 response, and that the action of the drug is compromised in some manner by the presence of HER3. Notably, biopsies that showed HER3 but not HER1 expression were less likely to respond to ABX-0303 (22% response rate) than patients whose tumors expressed both proteins (47% response rate). Dramatically, samples from patients that had low levels of HER3 but expressed
10   HER1 and/or pERK at levels of greater than 9, had a 100% response rate to ABX-0303. An analysis with a greater number of samples will help confirm any of the conclusions drawn from this analysis. Examples of tumors with high and low levels of HER3 are provided in Figures 1 and 2.

This data indicates that ABX-0303 effectively targets HER1 to prevent the growth
15   of renal cell cancers. It is not surprising that HER1 seems to be acting through the MAPK pathway, as shown by the positive correlation between pERK expression and response. Of interest is the role of HER3, which was found elevated in 79% of the renal biopsies analyzed.

As will be appreciated, the above findings provide useful methods for the
20   selection of patients for treatment with molecules that target EGFr and predictors of the response of patients. In addition, the above findings provide useful methods for the use of ABX-0303. ABX-0303 is described in detail in U.S. Patent No. 6,235,883 (the disclosure of which is hereby incorporated by reference) and referred to therein in connection with the discussions related to hybridoma E7.6.3.

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It should be understood that the foregoing disclosure emphasizes certain specific embodiments of the invention and that all modifications or alternatives equivalent thereto are within the spirit and scope of the invention as set forth in the appended claims. All references discussed herein are hereby incorporated by reference in their entirety.

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WHAT WE CLAIM IS:

1.   A method for predicting a response to an epidermal growth factor receptor-directed therapy in a human subject, the method comprising the step of assaying a tumor sample from the human subject before therapy with one or a plurality of reagents that detect expression and/or activation of predictive biomarkers for cancer; and determining a pattern of expression and/or activation of at least two of said predictive biomarkers, wherein the pattern predicts the human subject's response to the epidermal growth factor receptor-directed therapy.

2.   The method of claim 1, wherein the predictive biomarker is a growth factor receptor, or a growth factor receptor-related downstream signaling molecule.

3.   The method of claim 2, wherein the growth factor receptor is HER1 (EGFR), pHER1, HER2/neu, HER3, or any combination thereof.

4.   The method of claim 2, wherein the growth factor receptor-related downstream signaling molecules is pERK.

5.   The method of claim 2, wherein the growth factor receptor is HER1 (EGFR), pHER1, HER2/neu, HER3, or any combination thereof, and the growth factor receptor-related downstream signaling molecules is pERK.

6.   The method of claim 1, where in the predictive biomarkers are HER1 (EGFR) and HER3.

7.   The method of claim 6, wherein when HER1 (EGFR) is undetectable is predictive of the human subject not responding to the epidermal growth factor receptor-directed therapy.

8.   The method of claim 6, wherein when HER3 is undetectable is predictive of the human subject responding to the epidermal growth factor receptor-directed therapy.

9.   The method of claim 1, where in the predictive biomarkers are HER1 (EGFR) and pERK.

10.   The method of claim 1, where in the predictive biomarkers are pERK and HER3.

11.   The method of claim 1, where in the predictive biomarkers are HER1 (EGFR), HER3, and pERK.

12.   A kit for determining a response to an epidermal growth factor receptor-directed therapy in a subject comprising at least two reagents that detect expression and/or activation of predictive biomarkers for cancer.

13.   The kit of claim 12, wherein said kit comprises at least three reagents that detect expression and/or activation of predictive biomarkers for cancer.

14.   The kit of claim 12, where the predictive biomarkers are HER1, HER3, pERK or any combination thereof.

15.   The kit of claim 13, where the predictive biomarkers are HER1, HER3, pERK or any combination thereof.

16.   A method for predicting a response to a cancer therapy in a human subject, the method comprising the step of assaying a cell or tissue sample from the human subject before therapy with one or a plurality of reagents that detect expression and/or activation of predictive biomarkers for cancer, wherein said predicative biomarkers consist of growth factor receptor ligands; and determining a pattern of expression and/or activation of at least two of said predictive biomarkers, wherein the pattern predicts the human subject's response to the cancer therapy.

17.   The method of claim 16, wherein the growth factor receptors are HER1 (EGFR), pHER1, HER2/neu, HER3 or any combination thereof.

18.   The method of claim 17, wherein the cancer therapy is an epidermal growth factor receptor-directed therapy.

19.   The method of claim 18, wherein the cancer therapy is an anti-EGFR antibody.

20.   The method of claim 19, wherein the antibody is ABX-0303.

21.   A method of selecting a subject with cancer for treatment with a molecule targeting epidermal growth factor receptor (EGFR), comprising determining the level of expression of HER3 in a cell or tissue sample from the subject, wherein if the level of HER3 expression is low in the cells, the subject is selected.

22.   The method of claim 21, wherein the molecule is an anti-EGFR antibody.

23.   The method of claim 22, wherein the antibody is ABX-0303.

24.   The method of claim 21, wherein the determining step further comprises determining expression of one or more of HER1 (EGFR), pHER1, HER2/neu, and pERK.

25.   A method of predicting the likely response rate to a molecule targeting epidermal growth factor receptor (EGFR) of a subject having a cancer that overexpresses EGFR, comprising the step of determining the level of expression of HER3 in a cell or tissue sample from the subject, wherein if the level of HER3 expression is low in the cells, the subject is likely to respond to the molecule targeting EGFR.

26.   The method of claim 25, wherein the molecule is an anti-EGFR antibody.

27.   The method of claim 26, wherein the antibody is ABX-0303.

28.   The method of claim 25, wherein the determining step further comprises determining expression of one or more of HER1 (EGFR), pHER1, HER2/neu, and pERK.

29.   A method of treating a subject with cancer, comprising determining the level of expression of HER3 in the cells from the subject, and treating the subject with an anti-EGFR antibody when HER3 expression levels in the cell are low.

5   30.   The method of claim 29, wherein the antibody is ABX-0303.

31.   The method of claim 29 wherein the determining step further comprises determining expression of one or more of HER1 (EGFR), pHER1, HER2/neu, and pERK.

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32.   The method of claim 31, wherein the antibody is ABX-0303.

33.   The method of claim 29, wherein the level of expression of HER3 is undetectable.

15   34.   The method of claim 33, wherein the antibody is ABX-0303.

35.   A method of selecting a subject with cancer for treatment with a molecule targeting epidermal growth factor receptor (EGFR), the method comprising:

a)   determining an expression and/or activation profile of two or more growth
20   factor receptors in cells and/or tissues of the subject; and

b)   selecting the subject based on the expression and/or activation profile, wherein the subject is selected when the level of expression of HER3 is low, the level of expression of the HER1 is high, and/or the level of the pERK index is high.

25   36.   The method of claim 35, wherein the molecule is an anti-EGFR antibody.

37.   The method of claim 36, wherein the antibody is ABX-0303.

38.   The method of claim 37, wherein the growth factor receptors comprise one or
30   more of HER1 (EGFR), pHER1, HER2/neu, and HER3.

FIGURE 1
Patient 3019
Example of Elevated HER1 and pERK with low HER3

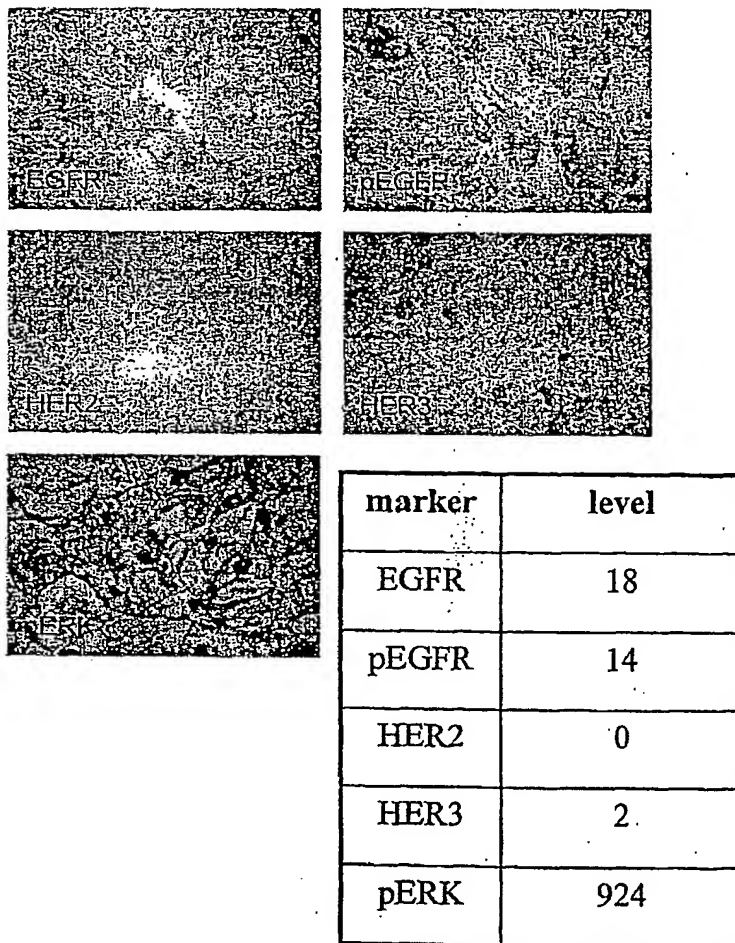| marker	level
EGFR	18
pEGFR	14
HER2	0
HER3	2
pERK	924

Figure 2
Example of Sample with Elevated HER1, HER3, and pERK

marker	level
EGFR	28
pEGFR	23
HER2	5
HER3	15
pERK	600